Bicyclophosphorothionate antagonists exhibiting selectivity for housefly GABA receptors†

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Abstract: 2,6,7-Trioxa-1-phosphabicyclo[2.2.2]octane 1-sulfides (bicyclophosphorothionates) with various $C_{1–4}$ alkyl groups at the 3- and 4-positions were synthesized and tested for their ability to compete with [^{3}H]4′-ethynyl-4-*n*-propylbicycloorthobenzoate (EBOB), a non-competitive antagonist of γ-aminobutyric acid (GABA) receptors, for specific binding to rat-brain and housefly-head membranes, and for their insecticidal activity against houseflies. Among the 3,4-substituted analogues, 20 compounds were selectively active for housefly GABA receptors versus rat GABA receptors. The 3-alkyl groups of C_3 length and the 4-alkyl groups of C_4 length were tolerated in housefly receptors, whereas such bulky substituents were deleterious in rat receptors. The 4-isobutyl-3-isopropyl analogue was the most potent in housefly receptors (IC_{50} = 45.2 nM), and *tert*-butylbicyclophosphorothionate (TBPS), with the 4-*tert*-butyl group and no 3-substituent, was the most potent in rat receptors (IC_{50} = 62.2 nM). Their receptor selectivities (rat IC_{50}/housefly IC_{50}) were 52 and 0.038, respectively. The insecticidal activity (LD_{50}) of 20 active analogues was well correlated with their potency (IC_{50}) in inhibiting [^{3}H]EBOB binding to housefly-head membranes (r = 0.93). The results obtained in the present study indicate that the introduction of appropriate alkyl groups into the 3- and 4-positions of bicyclophosphorothionate leads to non-competitive antagonists with increased affinity and selectivity for housefly ionotropic GABA receptors versus rat $GABA_A$ receptors.

Keywords: γ-aminobutyric acid; GABA receptor; non-competitive antagonist; bicyclophosphorothionate; TBPS; EBOB; insecticidal activity

1 INTRODUCTION

2,6,7-Trioxa-1-phosphabicyclo[2.2.2]octanes with certain substituents are potent convulsants and toxicants to mammals.[1] The 4-ethyl phosphate analogue was identified as a toxic substance produced in smoke on combustion of a non-commercial fire-retarded polyurethane foam.[2] Electrophysiological and biochemical studies have indicated that compounds of this type act as non-competitive antagonists of ionotropic γ-aminobutyric acid (GABA) receptors in both invertebrate and vertebrate nervous systems.[3–9]

In structure–toxicity relationship studies, 4-*tert*-butyl-2,6,7-trioxa-1-phosphabicyclo[2.2.2]octane 1-oxide (TBPO) was found to be outstandingly toxic to mice (intraperitoneal LD_{50} = 0.053 mg kg^{-1})[10] among this class of compounds.[11] The thiono analogue (*tert*-butylbicyclophosphorothionate, TBPS) of TBPO has been demonstrated to bind to rat brain $GABA_A$ receptors with high affinity, leading to the development of [^{35}S]TBPS as a radioligand for mammalian GABA-gated chloride channels ($GABA_A$ receptors).[6] In contrast, the *tert*-butyl analogues were not highly insecticidal, being comparable to other C_3 or C_4 alkyl analogues,[12] and TBPS was recently reported to be almost inactive or only weakly active in insect GABA receptors.[13,14] However, previous [^{35}S]TBPS binding and insecticidal assays have indicated that the introduction of a suitable alkyl group into the 3-position of the *n*-propyl analogue results in an increase in the affinity for insect GABA receptors.[15,16]

To explore further the effect of the 3-substituent, we synthesized more homologues and tested their affinity for housefly-head and rat-brain GABA receptors, using [^{3}H]4′-ethynyl-4-*n*-propylbicycloorthobenzoate (EBOB) established as a high-affinity ligand for non-competitive antagonist sites in both insect and mammalian GABA receptors, which gives a better correlation with insecticidal potency than the TBPS assay.[17] We here report that the introduction of

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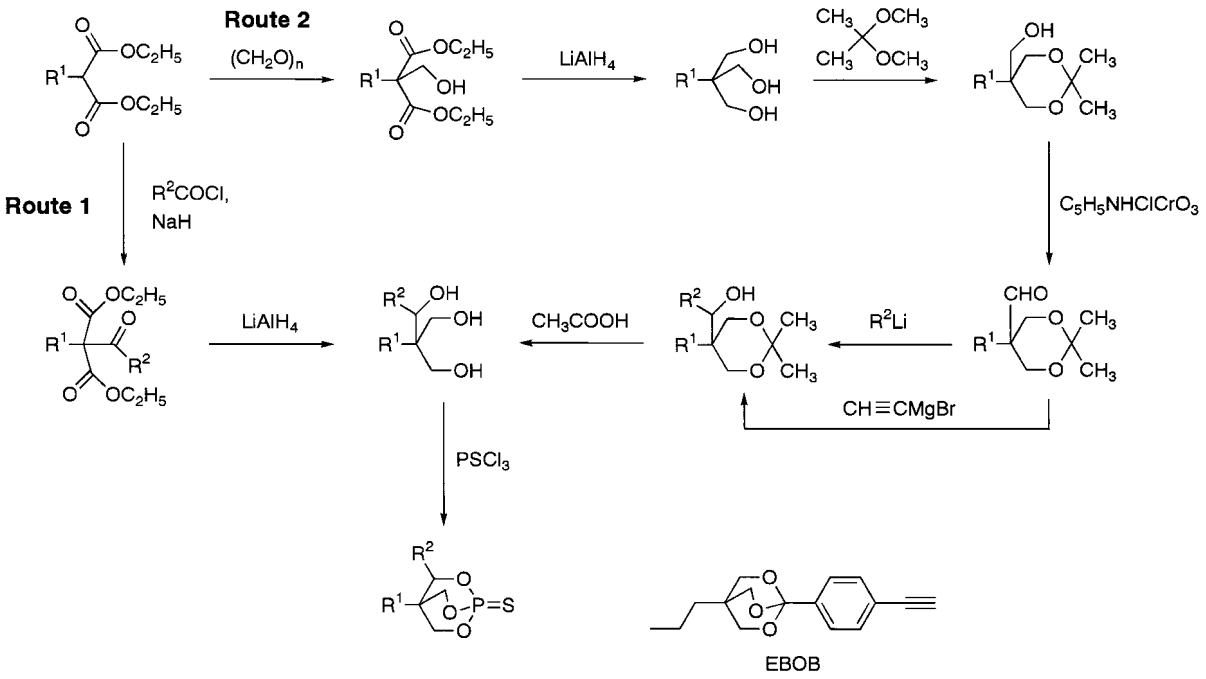


Figure 1. Routes for synthesis of 3,4-disubstituted bicyclophosphorothionates and structure of EBOB.

appropriate alkyl groups into the 3- and 4-positions of 2,6,7-trioxa-1-phosphabicyclo[2.2.2]octane 1-sulfide (bicyclophosphorothionate) leads to non-competitive antagonists with increased affinity and selectivity for housefly ionotropic GABA receptors versus rat $GABA_A$ receptors.

2 EXPERIMENTAL

2.1 Chemicals

[^{3}H]EBOB (1406 GBq mmol^{-1}) was purchased from NEN™ Life Science Products, Inc (Boston, MA). Compounds **1–9, 26** (TBPS), and EBOB were synthesized in our earlier studies.[15,18] Other bicyclophosphorothionates were synthesized by route 1 or route 2 as depicted in Fig 1, and their spectroscopic data are listed in Table 1. The synthesis of two typical compounds, **15** (route 1) and **17** (route 2), is described in Section 2.2.

2.2 Syntheses

2.2.1 General

Melting points were determined with a Yanako MP-500D apparatus. Melting and boiling points are uncorrected. [^{1}H] NMR spectra were obtained in deuterochloroform with a JEOL JNM-A400 (400 MHz) or JNM-GX270 (270 MHz) FT-NMR instrument with tetramethylsilane as an internal standard. Chemical ionization (CI) and high-resolution (HR) mass spectra (MS) were obtained on a Hitachi M-80B spectrometer.

*2.2.2 4-Isobutyl-3-isopropyl-2,6,7-trioxa-1-phosphabicyclo[2.2.2]octane 1-sulfide (**15**)*

2.2.2.1 Diethyl isobutyl(isobutyryl)malonate. A solution of diethyl isobutylmalonate (10.8 g, 50 mmol) in dry diethyl ether (30 ml) was added to a suspension of sodium hydride (1.2 g, 50 mmol) in dry ether (30 ml) dropwise with stirring at room temperature, and the mixture was then heated under reflux for 13 h and cooled to room temperature. To the cooled mixture was added a solution of isobutyryl chloride (5.3 g, 50 mmol) in dry ether (30 ml) dropwise with stirring, and the resulting mixture was refluxed for 15 h and diluted with water (30 ml) after cooling. The aqueous layer was extracted with ether (15 ml ×2). The combined ether extracts were dried (sodium sulfate) and concentrated under reduced pressure. The residual liquid was distilled under reduced pressure, yielding 9.6 g (67%) of diethyl isobutyl(isobutyryl)-malonate as a colourless liquid: bp 120–125 °C (5 mm Hg); MS (CI, isobutane) *m/z* 287 (M+1).

2.2.2.2 2-Hydroxymethyl-2-isobutyl-4-methyl-1,3-pentanediol. A solution of diethyl isobutyl(isobutyryl)malonate (9.6 g, 34 mmol) in dry ether (50 ml) was added dropwise to a suspension of lithium aluminum hydride (5 g, 132 mmol) in dry ether (50 ml) with stirring at 0 °C under a nitrogen atmosphere, and the mixture was refluxed for 24 h. The reaction mixture was allowed to cool to room temperature and added to a 10% sulfuric acid (150 ml) solution in an ice-bath. The aqueous layer was extracted with ethyl acetate (30 ml ×5), and the extract was dried (sodium sulfate) and concentrated under vacuum. The residue was purified by chromatography on silica gel with ethyl acetate+hexane (1+1 by volume) to afford 1.1 g (16%) of 2-hydroxymethyl-2-isobutyl-4-methyl-1,3-pentanediol as a colourless liquid: MS (CI, isobutane) *m/z* 205 (M+1).

*2.2.2.3 4-Isobutyl-3-isopropyl-2,6,7-trioxa-1-phosphabicyclo[2.2.2]octane 1-sulfide (**15**).* A solution of thiophosphoryl chloride (0.92 g, 5.4 mmol) in acetonitrile (5 ml) was added to a solution of 2-hydroxymethyl-2-isobutyl-4-methyl-1,3-pentanediol (1.1 g, 5.4 mmol) and pyridine (1.3 g, 16 mmol) in acetonitrile (50 ml) dropwise with stirring in an ice-bath, and the mixture was stirred for 30 min at room temperature. The acetonitrile was evaporated, and the residue was partitioned with water (60 ml) and chloroform (60 ml). The aqueous layer was extracted with chloroform (30 ml ×2), and the combined chloroform solutions were dried (sodium sulfate) and concentrated under vacuum. Recrystallization of the residue from ethanol provided 210 mg (15%) of **15** in the form of colourless needles (Table 1).

*2.2.3 4-tert-Butyl-3-isopropyl-2,6,7-trioxa-1-phosphabicyclo[2.2.2]octane 1-sulfide (**17**)*

2.2.3.1 Diethyl tert-butyl(hydroxymethyl)malonate. A mixture of diethyl *tert*-butylmalonate (43 g, 199 mmol), paraformaldehyde (37 g), potassium carbonate (7.2 g, 52 mmol), benzyltrimethylammonium chloride (0.5 g), and dimethyl sulfoxide (DMSO) (270 ml) was heated with stirring at 80 °C for 12 h.[10,19] After cooling to room temperature, the solution was diluted with water (600 ml) and extracted with chloroform (80 ml ×8). The chloroform extract was dried (sodium sulfate), concentrated under vacuum, and the residual liquid was distilled under reduced pressure, giving 16 g (33%) of diethyl *tert*-butyl(hydroxymethyl)malonate as a viscous colourless liquid: bp 107–110 °C (5 mmHg).

2.2.3.2 2-tert-Butyl-2-hydroxymethyl-1,3-propanediol. To a suspension of lithium aluminum hydride (6.4 g, 168 mmol) in dry ether (100 ml) was added a solution of diethyl *tert*-butyl(hydroxymethyl)malonate (14 g, 57 mmol) in dry ether (100 ml) dropwise at 0 °C under a nitrogen atmosphere. The mixture was refluxed for 36 h, added to a 10% sulfuric acid solution (200 ml) after cooling, and extracted with ether (100 ml ×8). The combined ether extracts were dried (sodium sulfate) and concentrated under vacuum. The residue was recrystallized from ether to give 5.1 g (55%) of 2-*tert*-butyl-2-hydroxymethyl-1,3-propanediol: mp 131–132 °C; MS (CI, isobutane) *m/z* 163 (M+1); [^{1}H]NMR δ 0.89 (9H, s), 2.95 (3H, s), 3.92 (6H, s).

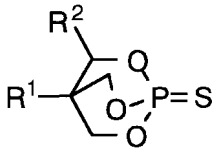
2.2.3.3 5-tert-Butyl-5-hydroxymethyl-2,2-dimethyl-1,3-dioxane. A solution of 2-*tert*-butyl-2-hydroxymethyl-1,3-propanediol (3 g, 18.5 mmol), 2,2-dimethoxypropane (1.9 g, 18.3 mmol), and *p*-toluenesulfonic acid monohydrate (133 mg, 0.70 mmol) in benzene (60 ml) was refluxed in a flask equipped with a Dean–Stark apparatus. After 12 h at reflux, the mixture was allowed to cool to room temperature and washed with a saturated sodium hydrogen carbonate solution. The benzene solution was dried (magnesium sulfate) and concentrated under reduced pressure. The residue was purified by chromatography on silica gel with diethyl ether + hexane (1 + 3 by volume) to give 2.9 g (78%) of 5-*tert*-butyl-5-hydroxymethyl-2,2-dimethyl-1,3-dioxane as a colourless solid: mp 46–47 °C; MS (CI, isobutane) *m/z* 203 (M+1); [^{1}H]NMR δ 0.93 (9H, s), 1.37 (3H, s), 1.39 (3H, s), 3.67 (2H, d, J = 12.0 Hz), 3.82 (2H, s), 3.91 (2H, d, J = 12.2 Hz).

2.2.3.4 5-tert-Butyl-5-formyl-2,2-dimethyl-1,3-dioxane. A solution of 5-*tert*-butyl-5-hydroxymethyl-2,2-dimethyl-1,3-dioxane (3.0 g, 14.9 mmol) in dry dichloromethane (30 ml) was added to a stirred suspension of pyridinium chlorochromate (9.3 g, 43 mmol) and anhydrous sodium acetate (0.6 g, 7.3 mmol) in dry dichloromethane (30 ml) at 0 °C under a nitrogen atmosphere. The mixture was stirred at room temperature for 6 h and then diluted with ether (10 ml), and the organic solution was decanted from the tarry precipitate, which was rinsed with ether (10 ml ×2). The combined organic solutions were evaporated under vacuum. The residue was purified by chromatography on silica gel with diethyl ether + hexane (1 + 3 by volume) to give 2.6 g (87%) of 5-*tert*-butyl-5-formyl-2,2-dimethyl-1,3-dioxane as a white solid: mp 41–42 °C; MS (CI, isobutane) *m/z* 201 (M+1); [^{1}H]NMR δ 0.96 (9H, s), 1.29 (3H, s), 1.41 (3H, s), 4.03 (2H, d, J = 12.2 Hz), 4.26 (2H, d, J = 12.0 Hz), 9.84 (1H, s).

2.2.3.5 5-tert-Butyl-5-(1-hydroxy-2-methylpropyl)-2,2-dimethyl-1,3-dioxane. A mixture of 5-*tert*-butyl-5-formyl-2,2-dimethyl-1,3-dioxane (0.5 g 2.5 mmol), isopropyl bromide (0.46 g, 3.7 mmol) and lithium pieces 0.07 g (0.01 g-atom) in dry tetrahydrofuran (THF) (12.5 ml) was sonicated for 3 h at 0 °C under a nitrogen atmosphere.[20] After the THF solution was allowed to warm to room temperature, the contents of the flask were filtered with suction through a Buchner funnel without filter paper to remove excess lithium pieces. To the ice-cold THF solution was added water (20 ml), and the mixture was extracted with ether (10 ml ×2). The combined ether extracts were washed with saturated brine (40 ml), dried (magnesium sulfate), and concentrated under reduced pressure. The residue was purified by chromatography on silica gel with diethyl ether + hexane (2 + 3 by volume) to give 0.5 g (82%) of 5-*tert*-butyl-5-(1-hydroxy-2-methylpropyl)-2,2-dimethyl-1,3-dioxane as a colourless solid: mp 48–49 °C; MS (CI, isobutane) *m/z* 245 (M+1); [^{1}H]NMR δ 0.98 (9H, s), 1.05 (3H, d, J = 7.1 Hz), 1.07 (3H, d, J = 6.8 Hz), 1.35 (3H, s), 1.37 (3H, s), 2.15 (1H, m), 3.58 (1H, s), 3.77 (1H, d, J = 12.2 Hz), 3.78 (2H, s), 3.92 (1H, d, J = 12.2 Hz).

2.2.3.6 2-tert-Butyl-2-hydroxymethyl-4-methyl-1,3-pentanediol. A mixture of 5-*tert*-butyl-5-(1-hydroxy-2-methylpropyl)-2,2-dimethyl-1,3-dioxane (0.45 g, 1.8 mmol), acetic acid (8 ml), and water (4 ml) was heated to 60 °C for 1 h with stirring and then cooled to room temperature. After addition of a saturated

Table 1. Structures and characterization data for new bicyclophosphorothionates



Comp No	R^1	R^2	Synthesis route	mp (°C)	HRMS (M^+) (m/z) Calc	HRMS (M^+) (m/z) Found	[^{1}H]NMR ($CDCl_3$) (ppm)
10	H	*i*-C_3H_7	1	150–151	208.0321	208.0317	0.91 (3H, d, J=6.8Hz, ($C\underline{H}_3$)$_2$CH), 1.17 (3H, d, J=6.8Hz, ($C\underline{H}_3$)$_2$CH), 2.20 (1H, m, (CH_3)$_2$C$\underline{H}$) 2.31 (1H, m, 4-C$\underline{H}$), 4.26 (1H, m, C$\underline{H}_2$O), 4.57– 4.61 (2H, m, C$\underline{H}_2$O), 4.70–4.76 (2H, C$\underline{H}_2$O, (CH_3)$_2$CHC$\underline{H}$)
11	CH_3	*i*-C_3H_7	1	109–110	222.0478	222.0470	0.90 (3H, s, C$\underline{H}_3$C), 1.11 (3H, d, J=6.8Hz, ($C\underline{H}_3$)$_2$CH), 1.16 (3H, d, J=6.6Hz, ($C\underline{H}_3$)$_2$CH), 2.13 (1H, m, (CH_3)$_2$C$\underline{H}$), 4.28 (4H, m, (C$\underline{H}_2$O)$_2$), 4.65 (1H, m, (CH_3)$_2$CHC$\underline{H}$)
12	C_2H_5	*i*-C_3H_7	1	113–114	236.0634	236.0607	0.88 (3H, t, J=7.7Hz, C$\underline{H}_3$CH$_2$), 1.14 (3H, d, J=7.1Hz, ($C\underline{H}_3$)$_2$CH), 1.17 (3H, m, ($C\underline{H}_3$)$_2$CH), 1.27–1.51 (2H, m, CH$_3$C$\underline{H}_2$), 2.13 (1H, m, (CH_3)$_2$C$\underline{H}$), 4.30–4.46 (4H, m, (C$\underline{H}_2$O)$_2$), 4.60 (1H, m, (CH_3)$_2$CHC$\underline{H}$)
13	*i*-C_3H_7	*i*-C_3H_7	1	93–94	250.0792	250.0795	0.92 (3H, m, ($C\underline{H}_3$)$_2$CHC), 1.14 (3H, d, J=7.1Hz, ($C\underline{H}_3$)$_2$CHCH), 1.18 (3H, d, J=6.6Hz, ($C\underline{H}_3$)$_2$CHCH), 1.19–1.32 (4H, m, ($C\underline{H}_3$)$_2$C$\underline{H}$C), 2.14 (1H, m, (CH_3)$_2$C$\underline{H}$CH), 4.30–4.45 (4H, m, (C$\underline{H}_2$O)$_2$), 4.60 (1H, m, (CH_3)$_2$CHC$\underline{H}$)
14	*n*-C_4H_9	*i*-C_3H_7	1	89–90	264.0948	264.0931	0.91 (3H, t, J=7.1Hz, C$\underline{H}_3$(CH$_2$)$_3$), 1.14 (3H, d, J=6.8Hz, ($C\underline{H}_3$)$_2$CH), 1.18 (3H, d, J=6.6Hz, ($C\underline{H}_3$)$_2$CH), 1.15–1.34 (6H, m, CH$_3$(C$\underline{H}_2$)$_3$), 2.12 (1H, m, (CH_3)$_2$C$\underline{H}$), 4.30–4.45 (4H, m, (C$\underline{H}_2$O)$_2$), 4.60 (1H, m, (CH_3)$_2$CHC$\underline{H}$)
15	*i*-C_4H_9	*i*-C_3H_7	1	105–106	264.0948	264.0954	0.93 (6H, d, J=6.6Hz, ($C\underline{H}_3$)$_2$CHCH$_2$), 1.10– 1.31 (2H, m, (CH_3)$_2$CHC$\underline{H}_2$), 1.12 (3H, d, J=6.8Hz, ($C\underline{H}_3$)$_2$CH), 1.16 (3H, d, J=6.6Hz, ($C\underline{H}_3$)$_2$CH), 1.60–1.67 (1H, m, (CH_3)$_2$C$\underline{H}$CH$_2$), 2.13 (1H, m, (CH_3)$_2$C$\underline{H}$), 4.36–4.47 (4H, m, (C$\underline{H}_2$O)$_2$), 4.57 (1H, m, (CH_3)$_2$CHC$\underline{H}$)
16	*s*-C_4H_9	*i*-C_3H_7	1	116–117	264.0948	264.0973	0.89 (3H, d, J=6.8Hz, CH$_3$CH$_2$(C$\underline{H}_3$)CH), 0.93 (4H, m, C$\underline{H}_3$CH$_2$(CH$_3$)CH), 1.15 (3H, d, J=7.3Hz, ($C\underline{H}_3$)$_2$CH), 2.15 (3H, d, J=6.8Hz, ($C\underline{H}_3$)$_2$CH), 1.43–1.58 (2H, m, CH$_3$C$\underline{H}_2$(CH$_3$)C$\underline{H}$), 2.15 (1H, m, (CH_3)$_2$C$\underline{H}$), 4.37–4.66 (5H, m, (C$\underline{H}_2$O)$_2$, (CH_3)$_2$CHC$\underline{H}$)
17	*t*-C_4H_9	*i*-C_3H_7	2	187–188	264.0948	264.0952	1.01 (9H, s, ($C\underline{H}_3$)$_3$C), 1.18 (3H, d, J=6.8Hz, ($C\underline{H}_3$)$_2$CH), 1.23 (3H, d, J=6.6Hz, ($C\underline{H}_3$)$_2$CH), 2.39 (1H, m, (CH_3)$_2$C$\underline{H}$), 4.39–4.56 (4H, m, (C$\underline{H}_2$O)$_2$), 4.80–4.85 (1H, m, (CH_3)$_2$CHC$\underline{H}$)
18	H	*s*-C_4H_9	1	123–124	222.0477	222.0475	0.87, 1.15 (3H, 2d, J=6.8, 6.6Hz, CH$_3$CH$_2$(C$\underline{H}_3$)CH), 0.95, 0.97 (3H, 2t, J=7.3, 7.6Hz, C$\underline{H}_3$CH$_2$(CH$_3$)CH), 1.02–1.44 (2H, m, CH$_3$C$\underline{H}_2$(CH$_3$)CH), 2.00 (1H, m, CH$_3$CH$_2$(CH$_3$)C$\underline{H}$), 2.30, 2.33 (1H, 2m, 4-C$\underline{H}$), 4.36 (1H, m, C$\underline{H}_2$O), 4.60 (2H, m, C$\underline{H}_2$O), 4.72 (2H, m, C$\underline{H}_2$O, CH$_3$CH$_2$(CH$_3$)CHC$\underline{H}$)
19	CH_3	*s*-C_4H_9	1	68–69	236.0634	236.0631	0.88, 0.92 (3H, 2s, C$\underline{H}_3$C), 0.96, 0.97 (3H, 2t, J=7.4, 7.4Hz, C$\underline{H}_3$CH$_2$(CH$_3$)CH), 1.08, 1.16 (3H, 2d, J=6.8, 6.8Hz, CH$_3$CH$_2$(C$\underline{H}_3$)CH), 1.40–1.98 (3H, m, CH$_3$C$\underline{H}_2$(CH$_3$)C$\underline{H}$), 4.24–4.70 (5H, m, (C$\underline{H}_2$O)$_2$, CH$_3$CH$_2$(CH$_3$)CHC$\underline{H}$)

Table 1. Continued

Comp No	R^1	R^2	Synthesis route	mp (°C)	HRMS (M^+) (m/z) Calc	HRMS (M^+) (m/z) Found	[^{1}H]NMR ($CDCl_3$) (ppm)
20	C_2H_5	*s*-C_4H_9	1	66–67	250.0791	250.0819	0.91 (3H, t, *J* = 7.2 Hz, $C\underline{H}_3CH_2C$), 0.96, 0.97 (3H, 2t, *J* = 7.4, 7.4 Hz, $C\underline{H}_3CH_2(CH_3)CH$), 1.10, 1.16 (3H, 2d, *J* = 7.1, 6.8 Hz, $CH_3CH_2(C\underline{H}_3)CH$), 1.14–1.36 (2H, m, $CH_3C\underline{H}_2C$), 1.42–1.94 (3H, m, $CH_3C\underline{H}_2(CH_3)C\underline{H}$), 4.32–4.64 (5H, m, $(C\underline{H}_2O)_2$, $CH_3CH_2(CH_3)CHC\underline{H}$)
21	*i*-C_3H_7	*s*-C_4H_9	1	96–97	264.0947	264.0941	0.86–0.94 (6H, m, $(C\underline{H}_3)_2CHC$), 0.94–1.02 (3H, m, $C\underline{H}_3CH_2(CH_3)CH$), 1.13, 1.37 (3H, 2d, *J* = 6.3, 6.3 Hz, $CH_3CH_2(C\underline{H}_3)CH$), 1.22–2.35 (4H, m, $(CH_3)_2C\underline{H}C$, $CH_3C\underline{H}_2(CH_3)C\underline{H}$), 4.40–4.78 (5H, m, $(C\underline{H}_2O)_2$, $CH_3CH_2(CH_3)CHC\underline{H}$)
22	*n*-C_4H_9	*s*-C_4H_9	1	62–63	278.1104	278.1104	0.877, 0.884 (3H, 2t, *J* = 7.7, 7.7 Hz, $C\underline{H}_3CH_2CH_2CH_2$), 0.96, 0.97 (3H, 2t, *J* = 7.4, 7.4 Hz, $C\underline{H}_3CH_2(CH_3)CH$), 1.11, 1.16 (3H, 2d, *J* = 6.8, 6.8 Hz, $CH_3CH_2(C\underline{H}_3)CH$), 1.24–1.96 (9H, m, $CH_3(C\underline{H}_2)_3$, $CH_3C\underline{H}_2(CH_3)C\underline{H}$), 4.35–4.65 (5H, m, $(C\underline{H}_2O)_2$, $CH_3CH_2(CH_3)CHC\underline{H}$)
23	*i*-C_4H_9	*s*-C_4H_9	1	83–84	278.1104	278.1124	0.94 (6H, d, *J* = 6.6 Hz, $(C\underline{H}_3)_2CHCH_2$), 0.96, 0.98 (3H, 2t, *J* = 7.3, 7.6 Hz, $C\underline{H}_3CH_2(CH_3)CH$), 1.14, 1.16 (3H, 2d, *J* = 6.8, 6.6 Hz, $CH_3CH_2(C\underline{H}_3)CH$), 1.10–1.96 (6H, m, $CH_3C\underline{H}_2(CH_3)C\underline{H}$, $(CH_3)_2C\underline{H}C\underline{H}_2$), 4.40–4.62 (5H, m, $(C\underline{H}_2O)_2$, $CH_3CH_2(CH_3)CHC\underline{H}$)
24	*s*-C_4H_9	*s*-C_4H_9	2	91–98	278.1104	278.1081	0.87–1.00 (9H, 2($C\underline{H}_3CH_2(CH_3)CH$), $CH_3CH_2(C\underline{H}_3)CHC$), 1.11–1.18 (3H, $CH_3CH_2(C\underline{H}_3)CHCH$), 1.40–1.96 (6H, m, 2($CH_3C\underline{H}_2(CH_3)CH$)), 4.37–4.77 (5H, m, $(C\underline{H}_2O)_2$, $CH_3CH_2(CH_3)CHC\underline{H}$)
25	*t*-C_4H_9	*s*-C_4H_9	2	147–148	278.1104	278.1107	0.98 (3H, t, *J* = 7.7 Hz, $C\underline{H}_3CH_2(CH_3)CH$), 1.00, 1.01 (9H, 2s, $(C\underline{H}_3)_3C$), 1.17, 1.22 (3H, 2d, *J* = 6.6, 6.8 Hz, $CH_3CH_2(C\underline{H}_3)CH$), 1.50–2.18 (3H, m, $CH_3C\underline{H}_2(CH_3)C\underline{H}$), 4.39–4.68 (4H, m, $(C\underline{H}_2O)_2$), 4.84–4.88 (1H, m, $CH_3CH_2(CH_3)CHC\underline{H}$)
27	*t*-C_4H_9	*t*-C_4H_9	2	191–192	278.1104	278.1076	1.03 (9H, s, $(C\underline{H}_3)_3C$), 1.22 (9H, s, $(C\underline{H}_3)_3C$), 4.33–4.62 (4H, m, $(C\underline{H}_2O)_2$), 4.71–4.77 (1H, m, $(CH_3)_3CHC\underline{H}$)
28	*t*-C_4H_9	$CH_2C{\equiv}CH$	2	164–165	260.0636	260.0663	1.01 (9H, s, $(C\underline{H}_3)_3C$), 2.18 (1H, s, $C\underline{H}{\equiv}C$), 2.90–3.03 (2H, m, $CH{\equiv}CC\underline{H}_2$), 4.41–4.77 (4H, m, $(C\underline{H}_2O)_2$), 4.92–4.97 (1H, m, $CH{\equiv}CCH_2C\underline{H}$)
29	*t*-C_4H_9	$C{\equiv}CH$	2	136–137	246.0478	246.0478	1.05 (9H, s, $(C\underline{H}_3)_3C$), 2.88 (1H, s, $C\underline{H}{\equiv}C$), 4.42–4.95 (4H, m, $(C\underline{H}_2O)_2$), 5.26–5.29 (1H, m, $CH{\equiv}CC\underline{H}$)
30	*t*-C_4H_9	$C(CH_3){=}CH_2$	2	202–203	262.0791	262.0779	1.00 (9H, s, $(C\underline{H}_3)_3C$), 2.08 (3H, m, $CH_2{=}(C\underline{H}_3)C$), 4.55–4.63 (3H, m, $(C\underline{H}_2O)_2$), 4.85–4.93 (1H, m, $C\underline{H}_2O$), 5.19–5.20 (1H, m, $CH_2{=}C(CH_3)C\underline{H}$), 5.25 (1H, m, $C\underline{H}_2{=}(CH_3)C$), 5.29 (1H, s, $C\underline{H}_2{=}(CH_3)C$)

The approximate ratios of diastereomers contained in products were estimated on the basis of the integration of NMR peaks as follows: **16** (6:7), **18** (3:7), **19** (1:1), **20** (5:6), **21** (1:1), **22** (1:1), **23** (4:5), **24** (not determined), and **25** (3:4).

sodium hydrogen carbonate solution (20 ml), the resulting mixture was extracted with ether (10 ml ×4). The combined ether extracts were dried (sodium sulfate) and evaporated under vacuum. The residue was purified by chromatography on silica gel with diethyl ether + hexane (1 + 1 by volume) to afford 0.30 g (80%) of 2-*tert*-butyl-2-hydroxymethyl-4-methyl-1,3-pentanediol as a colourless solid: mp 80–81 °C; MS (CI, isobutane) *m/z* 205 (M+1); [^{1}H]NMR δ 0.98 (9H, s) 1.07 (6H, d, $\mathcal{J}$ = 6.3 Hz), 2.23 (1H, m), 2.63 (3H, brs), 3.79 (1H, s), 3.85 (1H, d, $\mathcal{J}$ = 11.5 Hz), 4.02 (2H, 2s), 4.13 (1H, d, $\mathcal{J}$ = 11.5 Hz).

*2.2.3.7 4-tert-Butyl-3-isopropyl-2,6,7-trioxa-1-phosphabicyclo[2.2.2]octane 1-sulfide (**17**).* This compound was

obtained from 2-*tert*-butyl-2-hydroxymethyl-4-methyl-1,3-pentanediol (0.30 g, 1.5 mmol) in the form of colourless needles (0.15 g, 39%) using the method described in Section 2.2.2.3 (Table 1).

2.3 Biological assays

2.3.1 [^{3}H]EBOB binding assays

2.3.1.1 Preparation of P_2 membranes. The procedure of Deng *et al*[21] was modified to prepare P_2 membranes from housefly heads. Housefly heads were obtained by shaking dry-ice-frozen adults (*Musca domestica* L, WHO strain), passing heads through a 2-mm diameter sieve at dry-ice temperature, and collecting them on a 1-mm diameter sieve. The heads were homogenized in Tris-HCl buffer (10 mM; pH 7.5; 20 ml) containing sucrose (0.25 M) with a Teflon-glass homogenizer, and the homogenate was diluted two-fold and centrifuged at 500 *g* for 5 min. The supernatant was filtered through four layers of 64-μm mesh nylon screen and centrifuged at 25 000 *g* for 30 min. The pellets were resuspended in the buffer, allowed to stand in an ice-bath for 30 min, and recentrifuged at 25 000 *g* for 30 min. The pellets were suspended in sodium phosphate buffer (10 mM; pH 7.5) containing sodium chloride (300 mM) and used directly for binding assays after protein determination.[22]

Rat-brain P_2 membranes were prepared based on the procedure of Squires *et al.*[6] Briefly, male Wistar rats (five weeks old) were killed by cervical dislocation, and their whole brains were removed, homogenized in EDTA, (1 mM), and then centrifuged at 1 000 *g* for 10 min. The supernatant was centrifuged at 25 000 *g* for 30 min. The resulting precipitate was suspended in EDTA (1 mM), packed into cellophane tubing, and dialysed against distilled, deionized water in an ice-bath (1–2 litre, 2 h ×3). The inner suspension was then centrifuged at 25 000 *g* for 30 min, and the pellets were stored at −80 °C. On the day of binding experiments, the pellets were suspended in the same binding buffer as above.

2.3.1.2 Binding experiments. The procedures of Deng *et al*[21] and Cole and Casida[17] were modified to perform [^{3}H]EBOB binding experiments. A mixture of DMSO (4 μl), [^{3}H]EBOB (5 nM) in sodium phosphate buffer (10 mM; pH 7.5) containing sodium chloride (300 mM) (0.1 ml), and homogenates of housefly-head membranes (0.9 ml, 200 μg protein) was used for the determination of total binding. The DMSO was replaced with unlabeled EBOB in DMSO (1.25 mM; 4 μl) to determine non-specific binding, and different concentrations of test compounds in DMSO (4 μl) were substituted for the DMSO to determine the inhibition by compounds. These mixtures were incubated at 22 °C for 70 min, followed by vacuum filtration on GF/B filters and two 5-ml rinses with ice-cold buffer, using a Brandel M-24 cell harvester. The filters were subjected to radio-counting in toluene-Methyl Cellosolve-based scintillation fluid with a Beckman LS 6000 SE instrument. Experiments with rat-brain membranes (125 μg protein per tube) were performed using the same procedure, except that the incubation temperature and time were 37 °C and 90 min, respectively.

Each experiment was performed in duplicate and repeated at least twice. Concentration-inhibition curves were drawn with five or six concentrations of inhibitors below 10 μM. IC_{50} values were calculated by the Probit method.

2.3.2 Insecticidal assays

Bicyclophosphorothionates were topically applied with acetone (1 μl) on the dorsal surface of the thorax of adult female houseflies (*Musca domestica* L, WHO strain), three to five days after emergence, 18.0 (±0.5) mg per fly. Fifteen flies were used for each dosage. The cytochrome P450 mono-oxygenase inhibitor, piperonyl butoxide (PB, 10 μg) in acetone (1 μl) was applied topically to each fly 1 h before the application of bicyclophosphorothionates. The flies were supplied with sugar and water at 25 °C, and the mortality was determined after 24 h, unless otherwise noted. Experiments were repeated at least twice. LD_{50} values were calculated from the mean values, using the Probit method.

2.4 Comparative molecular field analysis (CoMFA)

The log ($1/IC_{50}$ (M)) values of a superposed set of bicyclophosphorothionates were analyzed by the CoMFA[23]/QSAR module of SYBYL 6.4[24] on a Silicon Graphics Indigo^2 workstation, as described previously.[25] Unless otherwise stated, default settings were used throughout. The starting geometries of bicyclophosphorothionates were constructed by the modification of the X-ray crystal structure of methyl-bicyclophosphate,[26] using the SYBYL standard values of bond lengths and angles, and fully optimized by the semi-empirical molecular orbital method PM3.[27–29] The atomic charges were calculated by the MNDO method,[27,30,31] with the conformation fixed. The molecules were superposed at their 1-, 2-, 3-, and 4-positions by the Fit Atoms procedure within SYBYL. The samples assayed actually contained at least two stereoisomers (Table 1), depending on the structures of bicyclophosphorothionates. In the present analysis, however, all chiral carbon atoms of these compounds were assumed to be in the *R* configuration.

3 RESULTS

3.1 3-Alkyl-4-*n*-propyl-2,6,7-trioxa-1-phosphabicyclo[2.2.2]octane 1-sulfides (1–9)

Figure 2 shows the potency of 3-alkyl-4-*n*-propyl-substituted bicyclophosphorothionates in inhibiting specific [^{3}H]EBOB binding to membranes prepared from rat brains and housefly heads. Eight and three of nine homologues possessed IC_{50} values below 10 μM in housefly and rat GABA receptors, respectively. The order of the 3-substituent in yielding high activity was $i\text{-}C_3H_7 \geq t\text{-}C_4H_9 > C_2H_5 \geq CH_3 \geq s\text{-}C_4H_9 > H \geq$

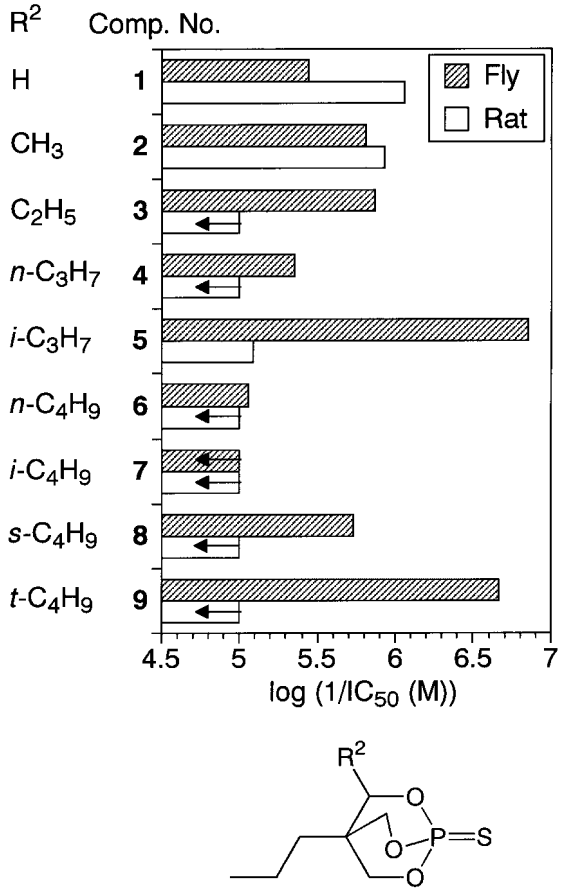


Figure 2. Inhibition of [^{3}H]EBOB binding by 3-alkyl-4-*n*-propyl-substituted bicyclophosphorothionates. Arrows indicate that IC_{50} values are more than 10 μM.

n-$C_3H_7 \geq$ *n*-C_4H_9 in housefly receptors and H $\geq$ CH_3 > *i*-C_3H_7 in rat receptors.

The introduction of an isopropyl or a *tert*-butyl group into the 3-position of **1** (NPPS) resulted in a marked increase in the activity in housefly GABA receptors. The IC_{50} values of **5** with the 3-isopropyl group and **9** with the 3-*tert*-butyl group were 142 nM and 220 nM, respectively (Table 2). In contrast, these compounds were weakly active or almost inactive in rat GABA receptors (8.07 μM and > 10 μM, respectively). Thus, **5** and **9** are highly selective inhibitors for housefly receptors versus rat receptors (receptor selectivity (rat IC_{50}/fly IC_{50}) = 56.8 and > 45.5, respectively).

3.2 4-Alkyl-3-isopropyl-2,6,7-trioxa-1-phosphabicyclo[2.2.2]octane 1-sulfides (5, 10–17)

Because, in the 3-alkyl-4-*n*-propyl series of compounds, the 3-isopropyl homologue showed the highest potency, we next investigated the potency of 4-alkyl-3-isopropyl-substituted bicyclophosphorothionates in inhibiting specific [^{3}H]EBOB binding. The 4-substituent yielded high activity in the order *i*-$C_4H_9 \geq$ *t*-$C_4H_9 \geq$ *s*-$C_4H_9 \geq$ *n*-$C_4H_9 \geq$ *n*-$C_3H_7 \geq$ *i*-C_3H_7 > C_2H_5 in housefly receptors (Fig 3). Homologues bearing C_3 or C_4 alkyl groups in the 4-position were all highly active, with a similar IC_{50} level, while they were weak inhibitors in rat receptors. The potency in rat receptors decreased in the order of *t*-C_4H_9 > *s*-C_4H_9 = *i*-C_4H_9 > *n*-$C_4H_9 \geq$ *n*-$C_3H_7 \geq$ *i*-C_3H_7.

Compound **15**, bearing the 4-isobutyl group, displayed the highest potency among all compounds in housefly receptors (IC_{50} = 45.2 nM) and high selectivity for housefly receptors versus rat receptors (rat IC_{50}/fly IC_{50} = 52.2) (Table 2). Compound **14**, bearing the 4-*n*-butyl group, exhibited the highest receptor selectivity (rat IC_{50}/fly IC_{50} = 55.5). Compound **17**, bearing the 4-*tert*-butyl group, showed a potency similar to that of **15** in housefly receptors, although its receptor selectivity was relatively low.

3.3 4-Alkyl-3-*sec*-butyl-2,6,7-trioxa-1-phosphabicyclo[2.2.2]octane 1-sulfides (8, 18–25)

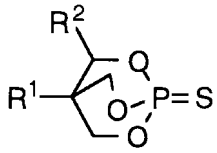
A previous study using [^{35}S]TBPS indicated that the introduction of the 3-*sec*-butyl group might lead to a ligand highly selective to insect GABA receptors.[16] Therefore, we investigated the potency of 4-alkyl-3-*sec*-butyl-substituted bicyclophosphorothionates in inhibiting [^{3}H]EBOB binding. All compounds with a *sec*-butyl group at the 3-position were almost inactive, with IC_{50} values of > 10 μM, in rat receptors (Fig 4). In contrast, the 3-*sec*-butyl homologues bearing a C_3 or C_4 4-substituent exhibited relatively high activity in housefly receptors. The rank order of potency for the 4-substituents was *i*-$C_4H_9 \geq$ *n*-$C_4H_9 \geq$ *s*-$C_4H_9 \geq$ *t*-C_4H_9 > *n*-C_3H_7 > *i*-C_3H_7 in housefly receptors.

The *sec*-butyl group at the 3-position was found to confer receptor selectivity to bicyclophosphorothionates, although the 3-*sec*-butyl compounds were less active than the 3-isopropyl compounds. Compound **23**, bearing the 4-isobutyl group, was most potent (IC_{50} = 256 nM) in housefly receptors and was most selective for housefly receptors (rat IC_{50}/fly IC_{50} > 39.1) in this series (Table 2).

3.4 3-Alkyl-, 3-alkenyl-, and 3-alkynyl-4-*tert*-butyl-2,6,7-trioxa-1-phosphabicyclo[2.2.2]octane 1-sulfides (17, 25–30)

In 3-alkyl, 3-alkenyl, and 3-alkynyl homologues of 4-*tert*-butyl-substituted bicyclophosphorothionates, the potency of the 3-isopropyl compound (**17**) was prominent in housefly receptors, and that of **26** (TBPS), with no substituent at the 3-position, in rat receptors (Fig 5). TBPS was weakly active in housefly receptors (IC_{50} = 1.62 μM), although it displayed the highest potency in rat receptors (IC_{50} = 62.2 nM). The absence of the 3-substituent makes bicyclophosphorothionate more selective for rat receptors than for housefly receptors (rat IC_{50}/fly IC_{50} of **26** = 0.0384) (Table 2). Compound **17**, with the 4-*tert*-butyl and 3-isopropyl groups, was among the most potent analogues in housefly receptors, with an IC_{50} value of 51.2 nM, but was not so selective for housefly receptors (rat IC_{50}/fly IC_{50} = 13.8) because of its relatively high activity (IC_{50} = 706 nM) in rat receptors. In contrast to the 4-*n*-propyl series, the replacement of

Table 2. Potency of bicyclophosphorothionates in inhibiting [^{3}H]EBOB binding to housefly-head and rat-brain membranes, and their synergized insecticidal activity against houseflies



Comp No	R^1	R^2	[^{3}H]EBOB binding IC_{50} (nM)[a]		RS[b]	Insecticidal activity LD_{50}[a] (μg per fly)
			Rat	Housefly		
1 (NPPS)	*n*-C_3H_7	H	873 (684–1110)	3650 (2650–5050)	0.239	0.147 (0.143–0.152)[c]
2	*n*-C_3H_7	CH_3	1170 (897–1530)	1540 (1160–2040)	0.760	0.138 (0.132–0.144)[c]
3	*n*-C_3H_7	C_2H_5	>10000	1340 (985–1820)	>7.46	0.221 (0.212–0.231)[c]
4	*n*-C_3H_7	*n*-C_3H_7	>10000	4490 (3020–6680)	>2.23	2.88 (2.52–3.30)[c,d]
5	*n*-C_3H_7	*i*-C_3H_7	8070 (5350–12200)	142 (107–189)	56.8	0.0681 (0.0646–0.0718)[c]
6	*n*-C_3H_7	*n*-C_4H_9	>10000	8740 (5060–15100)	>1.14	>10[c]
7	*n*-C_3H_7	*i*-C_4H_9	>10000	>10000	–	>10[c]
8	*n*-C_3H_7	*s*-C_4H_9	>10000	1850 (1350–2550)	>5.41	0.841 (0.799–0.886)[c,e]
9	*n*-C_3H_7	*t*-C_4H_9	>10000	220 (48.7–324)	>45.5	0.260 (0.235–0.288)[c]
10	H	*i*-C_3H_7	>10000	>10000	–	>10
11	CH_3	*i*-C_3H_7	>10000	>10000	–	>10
12	C_2H_5	*i*-C_3H_7	>10000	3340 (2410–4620)	>2.99	0.650 (0.538–0.784)
13	*i*-C_3H_7	*i*-C_3H_7	8170 (5270–12700)	208 (154–279)	39.3	0.117 (0.0966–0.141)
14	*n*-C_4H_9	*i*-C_3H_7	5120 (3530–7410)	92.2 (66.7–128)	55.5	0.101 (0.0816–0.123)
15	*i*-C_4H_9	*i*-C_3H_7	2360 (1810–3060)	45.2 (30.8–66.3)	52.2	0.0494 (0.0384–0.0635)
16	*s*-C_4H_9	*i*-C_3H_7	2360 (1760–3150)	81.8 (58.8–114)	28.9	0.0914 (0.0780–0.107)
17	*t*-C_4H_9	*i*-C_3H_7	706 (565–883)	51.2 (33.5–78.2)	13.8	0.0401 (0.0359–0.0449)
18	H	*s*-C_4H_9	>10000	>10000	–	>10
19	CH_3	*s*-C_4H_9	>10000	>10000	–	>10
20	C_2H_5	*s*-C_4H_9	>10000	>10000	–	>10
21	*i*-C_3H_7	*s*-C_4H_9	>10000	4210 (3010–5880)	>2.38	1.98 (1.69–2.31)
22	*n*-C_4H_9	*s*-C_4H_9	>10000	619 (427–899)	>16.2	0.524 (0.450–0.610)
23	*i*-C_4H_9	*s*-C_4H_9	>10000	256 (147–446)	>39.1	0.291 (0.251–0.338)
24	*s*-C_4H_9	*s*-C_4H_9	>10000	825 (581–1170)	>12.1	0.491 (0.409–0.589)
25	*t*-C_4H_9	*s*-C_4H_9	>10000	942 (673–1320)	>10.6	0.768 (0.623–0.945)
26 (TBPS)	*t*-C_4H_9	H	62.2 (49.8–77.7)	1620 (1180–2220)	0.0384	1.72 (1.47–2.02)
27	*t*-C_4H_9	*t*-C_4H_9	>10000	1670 (1160–2390)	>5.99	2.50 (1.70–3.69)
28	*t*-C_4H_9	$CH_2C{\equiv}CH$	763 (604–965)	332 (248–445)	2.30	0.621 (0.487–0.793)
29	*t*-C_4H_9	$C{\equiv}CH$	1380 (1080–1730)	2160 (1510–3100)	0.639	0.658 (0.540–0.801)
30	*t*-C_4H_9	$C(CH_3){=}CH_2$	2310 (1750–3050)	959 (703–1310)	2.41	1.35 (1.06–1.72)

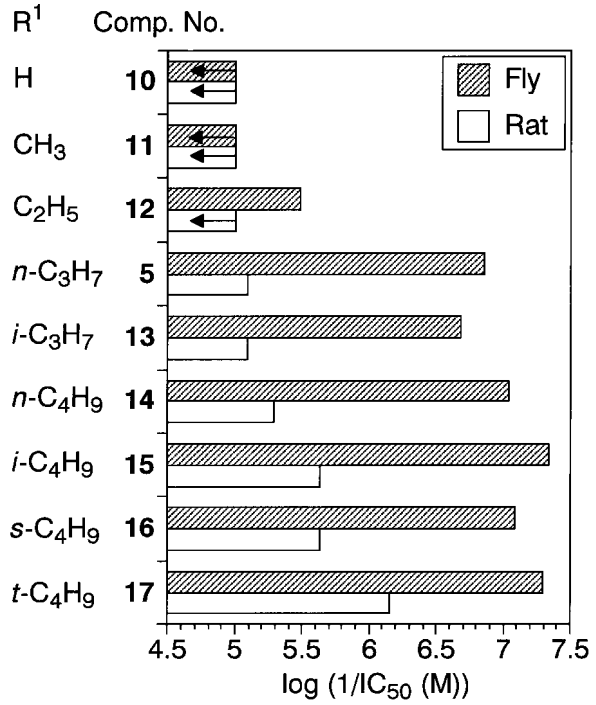
[a] Values in parentheses are 95% confidence limits.
[b] RS refers to receptor selectivity (rat IC_{50}/housefly IC_{50}).
[c] Taken from Ref 15.
[d] Determined at 48 h. 24-h LD_{50} was reported to be >10 μg per fly.[15]
[e] 48-h LD_{50} was reported to be 0.629 (0.594–0.665) μg per fly.[15].

the 3-isopropyl group with a *tert*-butyl group caused a marked decrease in potency for housefly receptors (**17** vs **27**). An unsaturated hydrocarbon chain (2-propynyl, ethynyl, or isopropenyl) at the 3-position was not effective in producing high affinity and selectivity for both receptors. It is noteworthy that **30** with the 3-isopropenyl group was approximately 30-fold less potent than **17** with the 3-isopropyl group in housefly receptors.

3.5 Correlation between in-vitro and in-vivo activities of bicyclophosphorothionates in houseflies

We have investigated the activity of 30 bicyclophosphorothionates for their inhibition of [^{3}H]EBOB binding to housefly-head membranes and their PB-synergized insecticidal activity to houseflies. Table 2 lists the IC_{50} values of bicyclophosphorothionates that showed more than 50% inhibition of [^{3}H]EBOB binding at 10 μM, as well as the LD_{50} values of bicyclophosphorothionates that showed more than 50% mortality at the dosage of 10 μg per fly. Twenty-four compounds had IC_{50} values of < 10 μM and 23 compounds LD_{50} values of < 10 μg per fly.

Figure 6 shows the relationship between IC_{50} and LD_{50} values of 23 compounds. Three compounds, **1**, **2** and **3**, appear to deviate from the correlation line of the other compounds; their actual insecticidal activities were higher than those expected from their IC_{50} values. Omitting the three compounds, 20 compounds



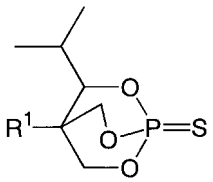
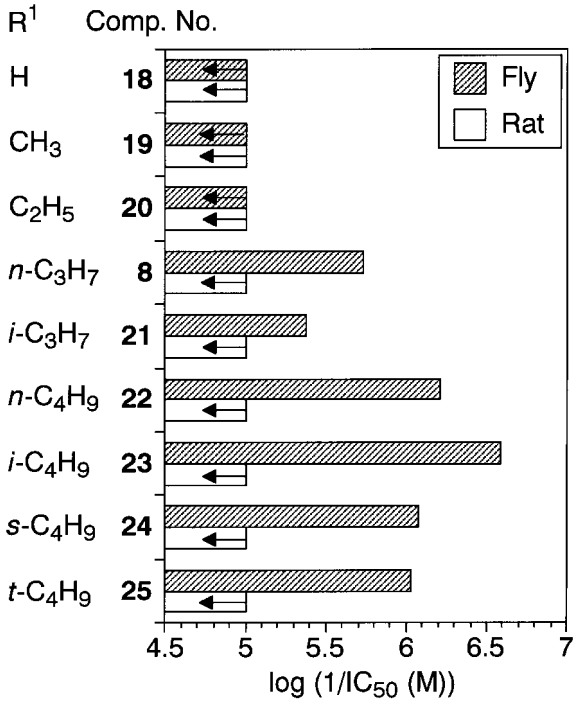


Figure 3. Inhibition of [^{3}H]EBOB binding by 4-alkyl-3-isopropyl-substituted bicyclophosphorothionates. Arrows indicate that IC_{50} values are more than 10 μM.



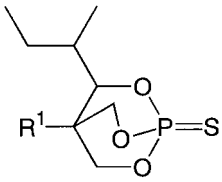


Figure 4. Inhibition of [^{3}H]EBOB binding by 4-alkyl-3-*sec*-butyl-substituted bicyclophosphorothionates. Arrows indicate that IC_{50} values are more than 10 μM.

showed a good correlation between in-vitro and in-vivo activities ($r = 0.93$).

3.6 Three-dimensional quantitative structure–activity relationships

To obtain quantitative information on the steric and electrostatic requirements for the selective action of bicyclophosphorothionates on housefly receptors, three-dimensional quantitative structure–activity relationship (3D-QSAR) analyses were attempted using the CoMFA procedure. The results are summarized in Table 3. All 24 active bicyclophosphorothionates were successfully included in the CoMFA analysis for housefly receptors, producing a satisfactory correlation, although the analysis for rat receptors was unsuccessful, probably because of the small number of active compounds and the narrow range of activity. Plate 1 displays a contour map of the CoMFA analysis for housefly receptors. The presence of the yellow contour outside the green contour around the 3-isopropyl group indicates that there is an optimum size of the *3*-substituent in the region. As for the area around the 4-substituent, there is no yellow contour, indicating that the 4-alkyl groups with more than four carbon atoms should be assayed to probe this area. The large blue contour might represent lipophilic interactions between the binding site and ligands.

4 DISCUSSION

In the present study we have demonstrated that the introduction of appropriate alkyl groups into the 3- and 4-positions of 2,6,7-trioxa-1-phosphabicyclo[2.2.2]octane 1-sulfide (bicyclophosphorothionate) leads to non-competitive antagonists with increased affinity and selectivity for housefly ionotropic GABA receptors versus rat $GABA_A$ receptors. In the case of 1-(4-ethynylphenyl)-2,6,7-trioxabicyclo[2.2.2]octanes (4′-ethynylbicycloorthobenzoates), bearing C_{3-4} *n*-alkyl groups at the 4-position, which were highly active in both housefly and mouse GABA receptors,[32] the introduction of a 3-cyano group into the 4-*tert*-butyl analogue has been reported to lead to a decrease in activity in insects, although it led to an increase in activity in mammals.[33,34] The present article is the first report that has described the enhanced selectivity of 3,4-disubstituted 2,6,7-trioxabicyclo[2.2.2]octanes for insect GABA receptors. Highly active analogues on housefly GABA receptors also showed a significant level of insecticidal activity against houseflies.

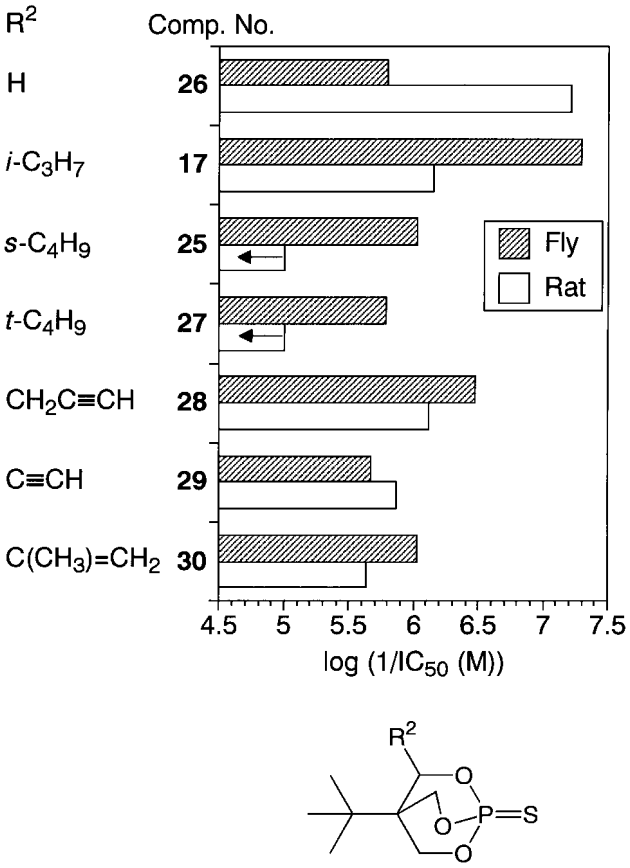


Figure 5. Inhibition of [^{3}H]EBOB binding by 3-alkyl-4-*tert*-butyl-substituted bicyclophosphorothionates. Arrows indicate that IC_{50} values are more than 10 μM.

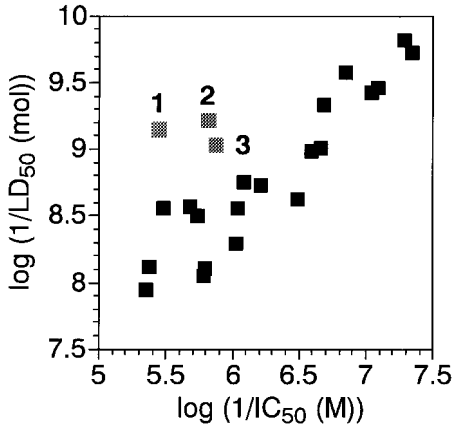


Figure 6. Correlation between the insecticidal activity (log ($1/LD_{50}$)) of bicyclophosphorothionates against houseflies and their potency (log ($1/IC_{50}$)) in inhibiting [^{3}H]EBOB binding to housefly-head membranes.

The substituent at the 3-position plays an important role in conferring the receptor selectivity to bicyclophosphorothionates. The optimal substituents for housefly receptors were the isopropyl group at the 3-position and the isobutyl group at the 4-position, as shown by **15**. Bicyclophosphorothionates that showed comparable potency included the analogues with the substituent combination of the 3-isopropyl and 4-*n*-propyl groups (**5**), the 3-*tert*-butyl and 4-*n*-propyl groups (**9**), the 3-isopropyl and 4-isopropyl groups (**13**), the 3-isopropyl and C_4 4-alkyl groups (**14, 16,** and **17**), and the 3-*sec*-butyl and 4-isobutyl groups (**23**). In contrast, such bulky alkyl groups were not tolerated in rat receptors. Compound **26** (TBPS), with the 4-*tert*-butyl group and no 3-substituent, was the only highly active analogue in rat receptors. CoMFA contour maps indicated the possibility that structural difference in the binding sites between insect and mammalian GABA receptors would be revealed if this method were applied to bicyclophosphorothionates with a variety of substituents and a wide range of activities against both receptors.

Table 3. Summary of CoMFA of bicyclophosphorothionate inhibition of [^{3}H]EBOB binding to housefly-head membranes

Number of compounds (*n*)	24
Optimum number of components	4
Standard error of estimate (*s*)	0.218
Square of correlation coefficient (r^2)	0.910
F value	49.908
Cross-validated standard error of predictions	0.475
Cross-validated r^2 (q^2)	0.571
Relative contribution–steric	0.938
Relative contribution–electrostatic	0.062

We have previously shown that picrotoxane terpenoids are also non-competitive antagonists selective for housefly GABA receptors.[35] Picrodendrin O, one of the terpenoids, displayed 76-fold selectivity for housefly receptors versus rat receptors. Also, fipronil, a phenylpyrazole insecticide, was reported to have an IC_{50} value of 2.3 or 6.3 nM in the inhibition of [^{3}H]EBOB binding to housefly-head membranes, and approximately 1,900-fold or 160-fold higher affinity for housefly receptors than for mouse receptors.[36,37] Rauh *et al*[38] also reported that a new class of GABA antagonists, polycyclic dinitriles, showed a significant level of selectivity for GABA receptors of southern corn rootworm (*Diabrotica undecimpunctata howardi* Barb). The potency and selectivity displayed by **14** and **15** are still low compared with those of fipronil. Non-competitive GABA antagonists, such as fipronil,[39] lindane and cyclodienes, contain nitrogen, sulfur or chlorine atom(s). In a future study, it would be interesting to observe how the activity and selectivity change if heteroatom-containing groups are introduced into the 3-, 4-, and other positions. It should also be noted that 3-substituted bicyclophosphorothionates comprise at least two stereoisomers, because a chiral center is introduced by the substituent at the 3-position. It remains to be determined which isomer is more active and selective.

To date, three genes that probably encode GABA receptor subunits have been cloned in the fruit fly (*Drosophila melanogaster* Meig).[40–42] The homologues of one (*Rdl*) of the three genes have been identified in many insect species, including the housefly, and the *Rdl*-encoded subunit of *Drosophila* was shown to form

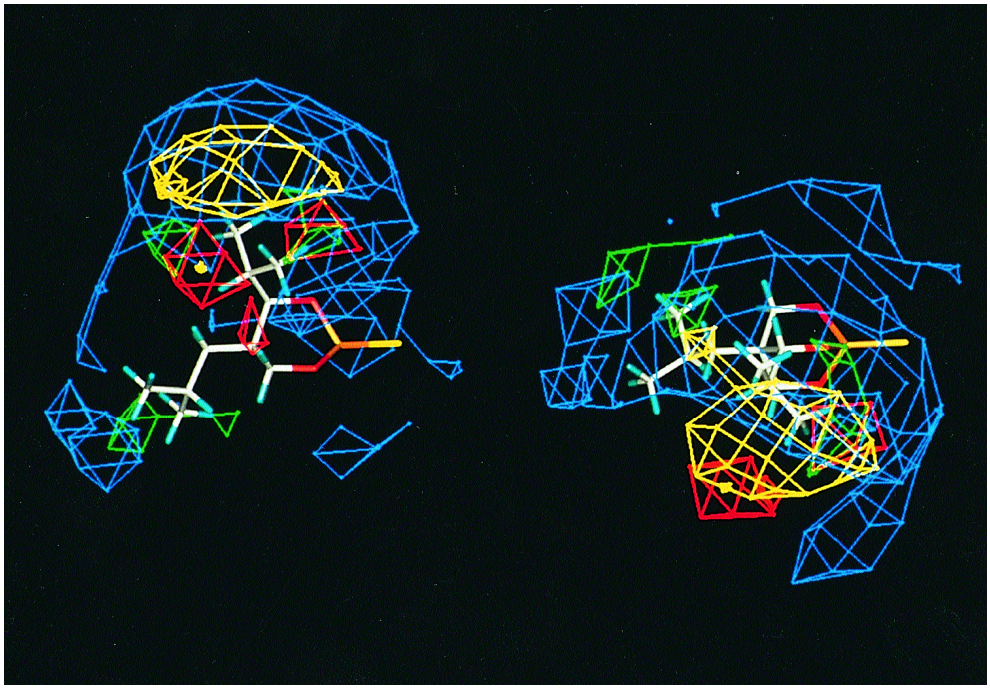


Plate 1. Orthogonal views of the steric and electrostatic CoMFA field maps with compound **15**. The green contours indicate regions of steric interaction that enhances affinity for receptors. The yellow contours are regions where steric interaction is unfavorable. The blue contours indicate regions in which positively charged moieties of a ligand increase receptor affinity. The red contours represent regions in which negatively charged moieties of a ligand enhance receptor affinity.

functional GABA-gated channels, indicating its contribution to the majority of insect GABA receptors.[43,44] Non-competitive antagonists are thought to interact with the channel-lining amino-acid residues of the second membrane-spanning region (M2) of GABA receptor subunits, which are highly conserved between *Rdl* and vertebrate β subunits.[40,44–46] While molecular cloning studies thus suggested that the structures of the binding sites might be homologous in insects and vertebrates, the current and previous structure–activity studies of various antagonists[25,35] suggest that there might be differences in the structures of the binding sites between rats and houseflies. Further clarification of the molecular basis of the receptor selectivity of non-competitive antagonists may have to await additional results from structure biological studies of GABA receptors. Nevertheless, comparative 3D-QSAR approaches, using ligands for insect and mammalian receptors as probes, may facilitate the development of safer insecticides.

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